REMARKS

The present application is directed to a method for detecting the presence of a target nucleic acid sequence in a sample by amplifying the target to produce an amplification reaction product that includes a purine rich region, contacting the sample during the amplification with a peptide nucleic acid able to bind to at least a portion of the target sequence and detecting the presence of triplex DNA structures. The application is also directed to a kit containing a bis-peptide nucleic acid (PNA) sequence designed to form a triplex with a target sequence and a set of amplification primers that can amplify a sequence including the target sequence.

Upon entry of the amendment, Claims 1-2, 5-6, 8-12, 18-19 and 22-26 will be pending. Claims 3-4, 7, 13-17, 20 and 21 have been cancelled without prejudice. Claims 1, 6, and 18 are currently amended. Support for the amendments can be found in the specification and claims as originally filed.

Rejection Under 35 U.S.C. 102(b)

The Examiner rejected Claims 1-2, 5-6, 8, 12, 22 and 24 under 35 U.S.C. § 102(b) as being anticipated by Ørum *et al.* (*Nucleic Acids Res.*, Vol. 21, No. 23, page 5332-5336, 1993). Applicants respectfully submit that the amendments to the claims overcome the rejection.

Applicants have amended independent Claims 1, 6 and 18 to clarify that the target sequence **comprises the purine rich region**. The purine rich region acts as a point of binding of the PNA probe, so that triplex structures are formed.

The Examiner concluded that Claims 1 and 6 are anticipated by Ørum *et al.* because some of the primers used by Ørum *et al.*, by coincidence, include regions containing four consecutive purine residues and are therefore categorized by the Examiner as "purine rich regions".

Applicants respectfully submit that Ørum *et al.* teach a method of PNA clamping to allow the detection of single base mutations. Specifically, Ørum *et al.* observed that PNA may be used to **block** amplification of a particular target sequence under particular circumstances

depending on the position of the PNA probe in relation to the amplification primer. Ørum *et al.* state that PNA will block amplification when it is used to compete for binding at the primer site, when it binds adjacent to the primer so that it blocks access for the polymerase or otherwise stops extension, as well as on occasions when it is arranged downstream of the primer site (see page 5334, column 1, lines 3-10 of text). The blocking is sufficiently clear for the technique to be used to determine the presence of a single base mutation. By selecting a PNA probe appropriately, the presence of a single base mutation may be detected by observing that amplification can be prevented. Thus, Ørum *et al.* teach that the **absence** of amplification products can be used to signal a single base mutation.

The Examiner stated that Ørum *et al.* discloses "triplex structures" in Figure 7 and on page 5335. Applicants respectfully submit that these sections of the Ørum *et al.* paper are directed to "PCR clamping". That is, where triplex structures are formed, the PCR reaction is **blocked** in a sequence-specific manner. It is clear from Figure 7 that **no** amplification products are observed when triplex structures are formed. In contrast, the pending claims are directed to the use of triplex structures to signal the **presence** of an amplification product.

The Examiner also stated that Ørum *et al.* teach the introduction of purine rich regions into the target sequence during amplification because some of the primers, specifically the "forward primer", "forward-1 primer" and "proximal primer", have four consecutive purines.

Applicants respectfully submit Ørum *et al.* did not deliberately select the primers named above because of the "four consecutive purines" characteristic. Indeed, looking at the primers cited by the Examiner, the "forward primer" is for the control pCKS sequence (see Figure 2), and it is clear from page 5333, column 1, lines 10-14, that the "forward primer" does "not contain a target sequence for any of the PNAs used in this study". Accordingly, applicants respectfully assert that the "forward primer" is not used in the context of the method set out in step (a) of pending Claims 1 and 6 because the amplification product it produces is **not able to bind to PNA**. With regard to the "forward-1 primer", applicants submit that the purine rich region of the "forward-1 primer" is not the target for PNA as is evident from Figure 7. The PNA clamp does not bind in the region of the forward-1 primer. Additionally, as discussed above, the limitations of steps (a) and (b) of Claims 1 and 6 are not met. Specifically, an amplification

product able to bind to a PNA is **not** produced. Instead, when PNA triplexes are formed, **no** target amplification occurs. With regard to the "proximal primer", this primer is arranged to fit adjacent to the PNA clamp, which works as intended and therefore **no amplification products are produced**. Applicants respectfully submit that the "proximal primer" does not fulfill the requirements of step (a) of Claims 1 and 6 because the target sequence is not amplified "wherein the resulting target sequence is able to bind to a peptide nucleic acid". Furthermore, the limitation of step (b) of Claims 1 and 6 is not met because "triplex structures resulting from the binding of the amplified target sequence to the peptide nucleic acid" are not present, rather triplex structures are **absent** due to clamping of the amplification.

Applicants respectfully submit that the instant claims represent a concept distinct to that of Ørum *et al.*, wherein the purpose of PNA and triplex in the instant application is not to **inhibit** amplification but to signal a **successful** amplification by acting effectively as a "label" on the amplification product. In contrast, Ørum *et al.* teach PCR clamping, which blocks PCR amplification and therefore **teaches away** from the claimed method.

Accordingly, applicants respectfully request withdrawal of the rejection of Claims 1-2, 5-6, 8, 12, 22 and 24 under 35 U.S.C. §102(b) as being anticipated by Ørum *et al.*

Rejection Under 35 U.S.C. 103(a)

In the Non-Final Office Action mailed October 16, 2007, the Examiner rejected Claims 9-11, 18-19, 23, 25-26 under 35 U.S.C. § 103(a) as obvious over Ørum *et al.* (as discussed above) in view of Graham *et al.* (WO 97/05280; hereinafter "Graham"). Applicants respectfully submit that amendments and remarks provided above with regard to the 35 U.S.C. 102(b) overcome the rejection.

Applicants have amended independent Claims 1, 16 and 18 to recite that the target sequence comprises the purine rich region. The dependent claims contain all the limitations of the base claim from which they depend. The claimed method comprises (a) amplifying the target sequence, **wherein the resulting target sequence is able to bind a peptide nucleic acid**; and (b) **detecting the presence of triplex structures resulting from the binding of the amplified**

target sequence to the peptide nucleic acid, wherein the detection of triplex structures indicates the presence of target nucleic acid sequences in the sample.

Applicants respectfully submit that the deficiencies of Ørum *et al.* are not satisfied by the teachings of Graham for at least the following reasons. Graham fails to teach or suggest a method for detecting the presence of a target nucleic in a sample by

(a) amplifying the target nucleic acid and introducing a purine rich region into the target sequence during the amplification, wherein the **resulting target sequence is able to bind a peptide nucleic acid**, and contacting the sample during the amplification with a peptide nucleic acid able to bind at least a portion of the target sequence comprising the purine rich region; and

(b) **detecting the presence of triplex structures resulting from the binding of the amplified target sequence to the peptide nucleic acid**,

wherein the detection of the presence of triplex structures indicates the presence of target nucleic acid sequences in the sample.

For at least the above reasons, applicants respectfully submit that the Ørum *et al.*, alone or in combination with Graham, fail to teach or suggest the claimed method and kit. Accordingly, applicants respectfully request withdrawal of the Examiner's rejection of Claims 9-11, 18-19, 23, 25-26 under 35 U.S.C. §103(a) as obvious over Ørum *et al.* in view of Graham.

CONCLUSION

In light of the amendments and the above remarks, applicants are of the opinion that the Office Action has been completely responded to and that the application is now in condition for allowance. Such action is respectfully requested.

If the Examiner believes any informalities remain in the application that may be corrected by Examiner's Amendment, or there are any other issues that can be resolved by telephone interview, a telephone call to the undersigned agent at (404) 815-6473 is respectfully requested.

No additional fees are believed due, however, the Commissioner is hereby authorized to charge any deficiencies that may be required, or credit any overpayment, to Deposit Account Number 11-0855.

Respectfully submitted,

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